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<p><b>(54) Title:</b> FUNCTIONALIZED POLYMER NANOPARTICLES, METHOD OF PREPARATION AND USE THEREOF</p> <p><b>(54) Titre:</b> NANOParticules de polymères fonctionnalisees, leur procédé de préparation et leur utilisation</p> <p><b>(57) Abstract</b> The present invention relates to polymer nanoparticles characterized in that they have a narrow grain size distribution, a size comprised between 10 et 50 nanometres and present at their surfaces identical or different ionogenic or reactive groupings. It also relates to the method for the preparation and use of said polymer nanoparticles in the coating industry and in biology.</p> <p><b>(57) Abrégé</b> La présente invention vise des nanoparticules de polymère caractérisées en ce qu'elles possèdent une distribution granulométrique resserrée, une taille comprise entre environ 10 et 50 nanomètres et présentent à leurs surfaces des groupements ionogènes ou réactifs, identiques ou différents. Elle se rapporte également à leur procédé de préparation et à leur utilisation dans l'industrie de revêtement et dans le domaine de la biologie.</p>			

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**FUNCTIONALIZED POLYMER NANOPARTICLES, METHOD OF  
PREPARATION AND USE THEREOF**

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**Abstract:** The present invention relates to polymer nanoparticles characterized in that that have a narrow grain size distribution, a size comprised between 10 and 50 nanometers and present at their surfaces identical or different ionogenic or reactive groupings. It also relates to the method for the preparation and use of said polymer nanoparticles in the coating industry and in biology.

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EP1245587A1 20021002 ROHM AND HAAS COMPANY

Improved coating and coating composition

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**Title in French:** NANOParticules de polymères fonctionnalisees, leur procédé de préparation et leur utilisation

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**Detailed Description**

## FUNCTIONALIZED POLYMER NANOPARTICLES, METHOD OF PREPARATION AND USE THEREOF

The present invention pertains to polymer particles of a size on the order of about 10 nanometers having on their surface ionogenic or reactive groups, the said particles occurring as such or in aqueous dispersions, as well as to the method of preparation and use thereof in biology.

The aqueous dispersions of polymer particles, more commonly called latices, are traditionally used in the coating, adhesives, paper and textile industries.

These dispersions are classically prepared by emulsion polymerization, a technique that makes it possible to prepare particles of a controlled size that is generally greater than  $0.1 \mu\text{m}$ . These qualities confer on them a large specific surface, which can be advantageously utilized for any application, taking into account the particular latex aspect.

The use of latices was recently extended to the area of biomedicine and more particularly to the immunological assays, such as diagnostic tests, the adsorption of proteins and the immobilization of enzymes. In this type of application, the latex particles classically carry on their surface reactive groups that are likely to be involved in the coupling reactions with biological molecules, e.g., antibodies.

Numerous studies are currently being conducted to increase the performance and more precisely the sensitivity of detection of these biological assay techniques. The size of the particles involved in the immunological assays is obviously one of the factors that determine their sensitivity threshold.

For example, the intensity of the light diffused by a given particle suspension depends on the number and the size of these particles in the optical assay such as nephelometry, which consists of detecting the light diffused by the aggregated particles via the formation of antibody-antigen complexes at a certain wavelength and at a given measuring angle. The smaller the size of the initial diffusing centers, the higher their concentration can be for the same diffused intensity. In the extreme case of particles with a very small diameter, on the order of 20 nm, their diffused intensity may be even negligible. Once the immunological reaction starts

and consequently once the particles associate in the form of doublets, triplets, etc., the light is diffused significantly, which makes possible the quantification of the reaction.

The object of the present invention is precisely to develop latex particles that have a more reduced size, i.e., on the order of about ten nanometers, and that are suitable for use in biological assays.

Considering the intended application, it is clear that the same polymer particles must also have a narrow particle size distribution to obtain a high sensitivity of detection and carry on their surface groups that are reactive to the biological molecules to be determined. It is particularly difficult to obtain these three characteristics at the same time, because it requires the use of functional polar monomers, which are partially soluble in water and which, moreover, tend to undergo polymerization in the aqueous phase; they may lead to the destabilization of the dispersion, which results in an increase in the mean particle size due to coalescence and in a considerable broadening of the particle size distribution.

The problem posed and solved according to the present invention is consequently the development of this novel type of latex particles which simultaneously meet three requirements, namely, reduced size, narrow particle size distribution and functionalization. More precisely, the present invention pertains to polymer nanoparticles characterized in that they have a narrow particle size distribution, a size between about 10 and 50 nanometers, and carry on their surface identical or different ionogenic or reactive groups.

The homopolymers or the copolymers containing repeat units derived from vinyl, acryl, or vinylaromatic monomers, from vinyl esters, alkyl esters of cc [sic] and p [sic] unsaturated acids, esters of unsaturated carboxylic acids, vinyl chloride, vinylidene chloride and/or dienes may be mentioned among the polymers that may form the said particles. More particularly, the following monomers may be mentioned as examples:

- Styrene and its derivatives (vinyl toluene, ethyl vinyl benzene),
- the esters, hydroxy esters and amides of (meth)acrylic acid, such as methyl methacrylate, butyl acrylate, and (meth)acrylamide,
- the vinyl esters (vinyl acetate, vinyl propionate),
- the vinyl and vinylidene chlorides,
- the vinylpyridines (2-vinylpyridine, 4-vinylpyridine, 2-methyl-5-vinylpyridine),
- the di(ethyl)aminoalkyl (meth)acrylates,
- the di(ethyl)aminoalkyl (meth)acrylamides,
- allylamine,

- ethyleneimine,
- (meth)acrylonitrile,
- *N*-vinylimidazole,
- the dialkylaminomethyl styrenes,
- vinylpyrrolidone,
- divinylbenzene and its derivatives,
- the conjugated dienes (butadiene),
- the polyallyl derivatives (tetraallyl ethylene),
- the (meth)acrylates of polyols (ethylene glycol dimethacrylate),
- methylene-bis(acrylamide),
- bis-(acrylamido)-acetic acid.

The compounds derived from styrene, acrylic acid, acrylic ester of the type of acrylic esters of *N*-hydroxysuccinimide, such as *N*-acryloyloxysuccinimide and *N*-acryloyloxyphthalimide, methacrylic acid, monobenzyl maleate, 2-vinylpyridine, styrene methyl sulfonate, chloromethylstyrene, hydroxypropyl methacrylate, hydroxybutyl acrylate, hydroxyethyl acrylate, acrylonitrile and/or acrolein may be mentioned more particularly as vinyl and acryl monomers that are suitable for use according to the present invention.

The ionogenic or reactive groups present on the surface of the particles are more particularly those mentioned above as substituents of the monomers from which the polymers forming the nanoparticles are derived.

They are preferably groups selected from among OH, SO<sub>3</sub>H, SO<sub>3</sub>R, SO<sub>4</sub>R, COOH, CHO, PhCH<sub>2</sub>Cl, NH<sub>2</sub>, NR<sub>2</sub>, NR<sub>3</sub>, in which R is a C<sub>1</sub>-C<sub>3</sub> alkyl radical, CONH<sub>2</sub>, NH-NH<sub>2</sub>, CN, CO<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>OH, in which n is an integer ranging from 1 to 8, as well as the esters of *N*-hydroxyimide.

The functional groups present on the surface of the nanoparticles may also be derived from the subsequent chemical conversion, e.g., by nucleophilic substitution, of one or more reactive groups carried by the polymer chain forming the nanoparticles.

According to a preferred aspect of the present invention, the polymer forming the nanoparticles has a glass transition temperature T<sub>g</sub> higher than about 20°C.

The nanoparticles according to the present invention have a narrow particle size distribution. This size monodispersity makes it possible to have access to the exact adsorption surface of the nanoparticles and consequently the optimal cation capacity of these nanoparticles in the immunological assays.

Any particle size distribution whose standard deviation is less than or equal to 30% and preferably on the order of 20% is considered to be narrow; this means that 2/3 of the particles on a weight basis have a diameter between  $d_{ma}$  and  $d_m + G$  ( $d_m$ : mean diameter, ( $Y$ ): standard deviation) in the case of a Gaussian distribution.

The nanoparticles according to the present invention may be obtained by the polymerization of a direct microemulsion of the corresponding monomer or monomers. A microemulsion is defined as a thermodynamically stable dispersion as opposed to an only kinetically stable dispersion, which will coalesce after a certain time. The present invention also pertains to the aqueous dispersions incorporating as particles the polymer nanoparticles according to the invention.

They are preferably aqueous dispersions containing about 1% to 25% and more particularly between about 5% and 20% of their weight of nanoparticles expressed as dry extract of the polymer forming the said particles.

The present invention also pertains to a method of preparation of the aqueous dispersions according to the invention.

The process according to the invention uses the polymerization of a direct microemulsion and the recovery of the said aqueous dispersion at the end of the polymerization, the said direct microemulsion being obtained in advance by means of an effective quantity of an ionic surfactant or a direct emulsion stabilized by at least one ionic surfactant or an inverse emulsion stabilized by at least one nonionic surfactant, the two emulsions, called initial emulsions, being formed by at least one monomer in an aqueous dispersion.

The aqueous nanoparticle dispersion thus obtained may also be subjected, if necessary, to a purification operation. The process consequently involves the formation of this direct microemulsion in a first step.

In the case of an inverse initial emulsion, the corresponding microemulsion is prepared

according to the inversion method suggested by the SCHULMAN process (*J. Phys. Chem.*, 1959, 63, p. 1677).

It consists of formulating at first a water-in-oil type emulsion, the oil being formed by the monomer or monomers being considered, by means of a nonionic lipophilic emulsifying agent, i.e., an emulsifying agent with a low hydrophilic-lipophilic balance HLB. The oil accounts for about 70 wt. % of the mixture. The quantity of this nonionic surfactant is added in such a way as to obtain a stabilized emulsion. In general, a small quantity of an anionic surfactant is added as well. Its quantity is approximately 0.5 wt. % relative to the weight of the monomer. This emulsion is subsequently inverted by the addition of an aqueous solution of a nonionic hydrophilic surfactant, i.e., a surfactant with a high HLB value, until the phase inversion is observed. This inversion phenomenon is macroscopically detectable because the change from a milky emulsion to a gel and then to a direct, stable, transparent microemulsion of low viscosity is consecutively observed in the course of one titration. The nonionic emulsifying agent used may be selected from among the nonyl phenol polyethoxylated derivatives (NPP). The lipophilic or hydrophilic character of these compounds varies as a function of the number of their ethoxy repeat units. Thus, the couples (NP5/NP15, NP15/NP12, NP7/NP12 and NP7/NP15) may be mentioned as couples formed by an ionic emulsifying agent with low HLB and a nonionic emulsifying agent with high HLB.

According to a preferred embodiment of the present invention, the inverse emulsion contains, in addition and prior to its titration, an anionic surfactant. It may be, e.g., sodium dodecylbenzene sulfonate (DBS), sodium lauryl sulfate (SDS) or sodium dioctyl sulfosuccinate (aerosol OT®).

The nanoparticle dispersions obtained according to this inverse emulsion technique contain a considerable quantity of surfactants and consequently cannot be used directly. The excess surfactants are subsequently removed according to a purification technique. This removal is preferably performed by dialysis, which has the advantage of not causing any change in the particle size. No flocculation phenomenon is seen.

The second technique, developed by the inventors for preparing the microemulsion, uses a direct emulsion as its starting point.

According to this method, the monomer or monomers are dispersed in an aqueous phase in

such a way as to obtain a direct emulsion containing additionally as a stabilizing agent an effective quantity of at least one ionic emulsifying agent. The weight percentages of oil and water are preferably on the order of 5% to 6%.

This emulsion is then titrated by the addition of a copolymerizable or noncopolymerizable co-surfactant until a translucent, oil-in-water type, stable direct emulsion of low viscosity is obtained. It is understood that the co-surfactant must be selected for its capacity to formulate a microemulsion from the direct emulsion and also for its ability to undergo joint micelle formation with the ionic emulsifying agent.

In particular, the A [sic] to C<sub>8</sub> straight-chain or branched aliphatic alcohols and preferably those derived from butyl alcohol or pentyl alcohol may be mentioned as possible noncopolymerizable co-surfactants, and the C<sub>2</sub> to C<sub>8</sub> hydroxyalkyl acrylate and hydroxyalkyl methacrylate derivatives may be mentioned as possible copolymerizable surfactants.

Cetyl trimethyl ammonium bromide, sodium dodecyl sulfate or sodium dodecyl benzene sulfonate may be used, in particular, as ionic emulsifying agents.

This second method of preparing the direct microemulsions is advantageous in several respects compared with the preceding technique.

In the first place, the quantities of emulsifying agent used are distinctly lower and, in the second place, the nature of these emulsifying agents does not give rise to consecutive problems in terms of removal. The alcohols of the pentyl alcohol type can be removed very easily by [unintelligible word] compared with the nonyl phenol polyethoxylated compounds.

In addition, the problem of removal does not arise in the case of the hydroxyalkyl acrylates or methacrylates because this emulsifying agent is incorporated by copolymerization in the structure of the polymer forming the nanoparticle formulation. Only the ionic emulsifying agent remains in the microemulsion in this case. Any need for a subsequent purification technique is also definitively eliminated by selecting a cationic surfactant such as cetyl trimethyl ammonium bromide (CTAB) or an anionic surfactant such as sodium lauryl sulfate (SDS) as an ionic emulsifying agent because CTAB and SDS precipitate at the end of the polymerization at ambient temperature and at a temperature below 15°C, respectively, and

they can consequently be easily removed by simple centrifugation or filtration.

Another advantage of this method of preparing the microlatices, which also applies to the so-called inversion method, is that the microemulsion can be obtained directly. Once the formulations have been established, the microemulsions can be prepared by directly mixing together the constituents. This ease of use is particularly interesting from an industrial viewpoint.

In general, the nanoparticles according to the invention have an emulsifying agent content below about 3%.

The microemulsions are preferably prepared at a temperature close to ambient temperature and always below the breaking temperature of the starting emulsion, which is typically on the order of 40°C.

The size of the particles of this microemulsion is on the order of 5 nm to 8 nm. The subsequent polymerization of these microemulsions by means of suitable initiators and under nondestructuring conditions makes it possible to obtain latices whose particle size is below 50 nm and which consequently have a narrow particle size distribution.

As far as this polymerization step of the microemulsions is concerned, its feasibility was established with water-soluble initiators and initiators soluble in organic solvents alike, which can be used at low temperatures, preferably below or equal to 40°C. They are redox couples (persulfate/diamine, hydrogen peroxide/ascorbic acid) or FAIBN or one of these water-soluble derivatives or DMPA (dimethoxyacetophenone), all being photochemically degradable (UV irradiation). Depending on the nature of the initiator system and the reaction temperature, the polymerization time is typically between 1 and 3 hours. As these polymerization techniques, called thermal, photochemical or chemical polymerization techniques, are very familiar to the person skilled in the art, they will not be described in detail here.

The homopolymerization or copolymerization reactions lead to particles with a narrow particle size distribution and a size on the order of 50 nm. They may be film-forming or non-film-forming particles. The monomers that can be used to prepare the nanoparticles are those already mentioned above.

The nanoparticles according to the present invention can be advantageously used in the biological assay methods for binding or immobilizing biologically active substances (proteins such as antibodies, enzymes, 2o [sic] antigens, drugs, etc.) by adsorption or coupling. The coupling reaction may be carried out according to well-known methods, e.g.,

- by using coupling agents such as glutaraldehyde, water-soluble carbodiimide, *N*-hydroxybenzotriazole, spacers of the 1,6-diaminohexane type, polysaccharide, etc., by activating the functional groups of the polymer (e.g., by diazotization, by the action of cyanogen bromide, tosyl chloride, etc.) and subsequent reaction with the molecule to be bound;
- by the direct reaction of the functional groups of the polymer, e.g., the activated acrylic ester groups of *N*-hydroxysuccinimide with amino derivatives (enzyme, amino acid, amino sugar, etc.) in the suspension at a pH close to the neutral value;
- by a nucleophilic substitution reaction with the surface chloromethyl groups, always directly in the suspension at neutral pH in the case of the anionic nucleophils (sulfite, thiocyanate, etc.) or at alkaline pH in the case of the amines (primary amines, amino acids, amino alcohols, diamines, etc.);
- etc.

These nanoparticles may also be used as a substrate in diagnostic tests ("RIA" [radioimmunological assay] agglutination, "IRMA" or immunoradiometric assay, "EIA" or enzyme immunoassay, "ELISA" or enzyme linked immunosorbent assay), as an enzyme catalyst in biotechnology or as a cell culture substrate. Considering their very small size, the nanoparticles and the corresponding aqueous dispersions may, of course, also be used advantageously in more industrial fields, such as the coating industry, e.g. painting, in the adhesives, textile and paper industries, etc.

The present application pertains to all applications of the nanoparticles being claimed, including, more particularly, those in biology and medicine, including the biological assay methods.

The examples presented below will permit other advantages and characteristics of the present invention to be demonstrated without limiting its scope.

The latices obtained in the examples below were characterized from the viewpoint of the particle size distribution either by PCS ("Photon Correlation Spectroscopy;" Quasi-elastic light diffusion) or by TEM (Transmission Electron Microscopy) and from the viewpoint of the determination of the surface functional groups, by conductimetric assay of surface functions or by UV test in the case of imide functional groups.

Some of them were also characterized by infrared and NMR spectroscopy if permitted by their solubility and by ultimate analysis in the case of the copolymers containing, e.g., halogens.

[Examples omitted from translation.—*The Language Service.*]

## CLAIMS

1. Polymer nanoparticles characterized in that they have a narrow particle size distribution, a size between about 10 nanometers and 50 nanometers and have identical or different ionogenic or reactive groups on their surface.
1. [sic; 2] Nanoparticles in accordance with claim 1, characterized in that the polymer forming the particles has a glass transition temperature Tg higher than about 20°C.
3. Nanoparticles in accordance with claim 1 or 2, characterized in that the polymer forming them is a homopolymer or copolymer containing repeat units derived from vinyl, acrylic, vinylaromatic monomers, from vinyl esters, alkyl esters of [unintelligible word] unsaturated acids, esters of unsaturated carboxylic acids, vinyl chloride, vinylidene chloride and/or dienes.
- [4.] Nanoparticles in accordance with claim 3, characterized in that they are preferably a polymer containing repeat units derived from the homopolymerization or copolymerization of styrene, acrylic acid, acrylic ester of the type of acrylic esters of *N*-hydroxysuccinimide, such as *N*-acryloyloxsuccinimide and *N*-acryloyloxyphthalimide[,] methacrylic acid, monobenzyl

maleate, 2-vinylpyridine, styrene methyl sulfonate, chloromethylstyrene, hydroxyethyl acrylate, hydroxypropyl methacrylate, [poly?]hydroxybutyl acrylate, acrylonitrile and/or acrolein.

5. Nanoparticles in accordance with any of the claims 1 through 4, characterized in that the ionogenic or reactive groups are preferably represented by at least one group selected from among OH, SO<sub>3</sub>H, SO<sub>3</sub>R, SO<sub>4</sub>R, COOH, CHO, PhCH<sub>2</sub>Cl, NH<sub>2</sub>, NR<sub>2</sub>, NR<sub>3</sub>, in which R is a C<sub>1</sub> and [sic] C<sub>3</sub> alkyl radical, CONH<sub>2</sub>, NH-NH<sub>2</sub>, CN, CO<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>OH, in which n is an integer ranging from 1 to 8, as well as esters of N-hydroxyimide.

6. Nanoparticles in accordance with any of the claims 1 through 5, characterized in that they have an emulsifying agent content below about 3%.

7. Nanoparticles in accordance with any of the above claims, characterized in that they can be obtained by the polymerization of a direct microemulsion of the corresponding monomer or monomers.

8. Aqueous polymer nanoparticle dispersion characterized in that it contains about 1% to 25% and preferably 5% to 20% of its weight of nanoparticles in accordance with one of the claims 1 through 7, expressed as the dry extract of the polymer forming the said particles.

9. Method of preparation of an aqueous dispersion in accordance with claim 8, characterized in that the polymerization of a direct microemulsion is carried out and the aqueous dispersion is recovered at the end of the polymerization, the said direct microemulsion having been obtained in advance by titration, by means of an effective quantity of a suitable surfactant, either

- of a direct emulsion stabilized by at least one ionic surfactant or
- of an inverse emulsion stabilized by at least one nonionic surfactant, the two emulsions, called initial emulsions, being formed by at least one monomer in an aqueous dispersion.

10. Method in accordance with claim 9, characterized in that the aqueous nanoparticle dispersion is subjected to a purification operation after the recovery.

11. Method in accordance with claim 9 or 10, characterized in that the 2-5 [sic] direct microemulsion is preferably prepared by the titration of a direct emulsion also containing at

least one ionic emulsifying agent besides the monomer or monomers by means of a copolymerizable or noncopolymerizable surfactant.

12. Method in accordance with claim 11 [sic; 11?], characterized in that the noncopolymerizable surfactant is preferably a straight-chain or branched C<sub>2</sub> to C<sub>8</sub> aliphatic alcohol and preferably the alcohols derived from butyl alcohol or pentyl alcohol.

13. Method in accordance with claim 11, characterized in that the copolymerizable surfactant is a C<sub>2</sub> to C<sub>8</sub> hydroxyalkyl acrylate or hydroxyalkyl methacrylate and preferably hydroxypropyl methacrylate.

14. Method in accordance with one of the claims 9 through 13, characterized in that the suitable surfactant is added to the emulsion until a stable and transparent direct microemulsion is obtained.

15. Method in accordance with one of the claims 9 through 14, characterized in that the inverse initial emulsion contains about 70 wt. % of monomer(s) and the direct emulsion contains about 5 wt. % to 6 wt. % of monomer(s).

16. Method in accordance with one of the claims 9 through 15, characterized in that the monomer or monomers present in the initial emulsion are preferably selected from among the monomers listed in claim 4.

17. Method in accordance with one of the claims 1 through 16, characterized in that the polymerization of the direct microemulsion is carried out photochemically, chemically or thermally by means of a suitable water-soluble initiator or a suitable initiator soluble in organic solvents.

18. Method in accordance with claim 17, characterized in that the initiator is preferably a redox couple of the persulfate/diamine type, hydrogen peroxide/ascorbic acid, AIBN or one of the water-soluble derivatives thereof.

19. Use of the nanoparticles in accordance with V [sic] one of the claims 1 through 7 or of an aqueous dispersion in accordance with claim 8 in the coating (paint), adhesives, textile and paper industries.

21. [sic; 20?] Use of the nanoparticles in accordance with one of the claims 1 through 7 or of an aqueous dispersion in accordance with claim 8 in biology and more particularly in biological assay methods.